EVIDENCE THAT THE STIMULATION OF PRECURSOR INCORPORATION INTO RNA OF RAT KIDNEY BY ALDOSTERONE IS MAINLY AN EFFECT ON UPTAKE

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1. Introduction

The effect of aldosterone on Na^+ retention by kidneys of adrenalectomized rats is blocked by inhibitors of RNA and protein synthesis [1–3]. Previous workers [2, 4, 5] have also shown that aldosterone stimulates the incorporation of radioactive precursors into RNA and this has been interpreted as meaning that RNA synthesis is stimulated. However it has become increasingly clear that hormones can modify pool sizes [6] and the rates of precursor uptake [7–9]; consequently the rate of incorporation of exogenous precursors is not necessarily a valid measure of the rate of RNA synthesis. We have therefore examined the effect of adrenalectomy and of aldosterone treatment on precursor uptake and acid soluble pools as well as its effect on the incorporation of precursors into acid insoluble material. The results obtained indicate that most of the stimulatory effect on the incorporation of exogenous precursor into RNA of rat kidney is attributable to changes in the rate of precursor uptake although a genuine effect on RNA synthesis cannot be ruled out.

2. Materials and methods

Male Sprague-Dawley rats (120–140 g) were adrenalectomized 7–8 days prior to the experiments and maintained on 1% sodium chloride and Purina Laboratory Chow ad libitum. Aldosterone was obtained from E.M. Reagent Div. Brinkmann Instruments Inc. Westbury, N.Y. 5-[^{3}H]Cytidine (specific activity 1 Ci/mmole) and 6-[^{14}C]orotic acid (specific activity 60.8 mCi/mmole) were purchased from Amersham/Searle, Arlington Heights, Illinois. The animals were divided into three groups; normal, adrenalectomized and adrenalectomized plus aldosterone treated. The animals were injected intraperitoneally with either aldosterone (5 μg/100 g body weight) or with saline 1 or 2 hr prior to the tracer injection. In one series of experiments each animal received an intraperitoneal injection of [^{3}H]cytidine (25 μCi/100 g body weight) 20 min before sacrifice. In another series each animal received an intraperitoneal injection of [^{14}C]orotic acid (5 μCi/100 g body weight) 20 min before sacrifice. Separate experiments showed that the incorporation of both isotopes into both RNA and the acid soluble pool was linear for 20 min but not beyond this time.

To isolate the acid soluble pool and the RNA fraction, the following procedure was employed. Animals were sacrificed by cervical dislocation at the designated time. Kidneys were removed quickly and frozen in liquid nitrogen. All subsequent steps were carried out at 0–4° unless otherwise indicated. Frozen kidneys were homogenized in Tris KCl-$MgCl_2$ buffer, pH 7.4 [10]. 0.2 ml of 50% trichloroacetic acid was added to a sample (0.8 ml) of homogenate, which was then diluted with 3.5 ml of 5% trichloroacetic acid and centrifuged to give acid soluble and acid insoluble fractions. The acid insoluble fraction was washed twice with 2 ml of 5% trichloroacetic acid, and the supernatants were combined to give the acid soluble

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fraction. The lipids were then removed from the acid insoluble fraction by ethanol, chloroform–ethanol, and ether washes [10]. The acid-insoluble defatted residue was hydrolyzed in 0.3 M KOH at 37° for 16 hr and after acidification with trichloroacetic acid, the soluble portion of this alkaline hydrolysate was assayed for radioactivity and for RNA content by the orcinol method [11]. The DNA was isolated from 0.1 ml of homogenate by the Schneider method [12] and determined with diphenylamine reagent [13].

Total radioactivity was determined on 0.1 ml aliquots of homogenate, solubilized with 0.4 ml of Protosol (New England Nuclear, Boston) in a glass counting vial at 55° for 2 hr. The vials were brought to room temp. and the pH was adjusted to between 6 and 7 with 0.02 ml conc. HCl. Samples were counted after adding 15 ml Aquasol (New England Nuclear, Boston). For measurement of radioactivity in the acid soluble pool and the alkaline hydrolyzate fraction, 0.2 ml aliquots of these fractions were counted in Aquasol. All radioactive samples were counted in a liquid scintillation spectrophotometer (Intertechnique Model SL30).

3. Results and discussion

Tables 1 and 2 show the effect of adrenalectomy and aldosterone treatment on the incorporation of [^{3}H]cytidine and [^{14}C]orotic acid into different fractions of rat kidney. There is a decrease in incorporation of precursors into the alkaline hydrolysate fraction after adrenalectomy. No decrease in incorporation into the acid soluble pool was observed.

Administration of aldosterone either 1 or 2 hr before the isotope results in an increased incorporation of precursor into macromolecular RNA. However, there is also a marked stimulation of uptake of precursor into the acid soluble pool. This increased uptake is sufficient to account for at least 65% of the stimulation of [^{3}H]cytidine and 45% of the stimulation of [^{14}C]orotic acid incorporation. There may also be a genuine increase in RNA synthesis but its magnitude is less than would be assumed if the acid insoluble incorporation alone were measured.

Measurements of the total acid soluble nucleotide pool showed that adrenalectomy and hormone treatment did not cause any significant changes. Values of

Table 1

Effect of adrenalectomy and aldosterone treatment on the incorporation of [^{14}C]orotic acid into different fractions of rat kidneys.

Fraction	[a]Normal$_{(5)}$	[a]Adrenalectomized$_{(6)}$	Difference (%)	[a]Adrenalectomized plus aldosterone treated		Difference (%)	
				1 hr$_{(4)}$	2 hr$_{(5)}$	1 hr	2 hr
Total counts (cpm/mg DNA)[b] $\times 10^{-4}$	17.35 ± 1.59	15.01 ± 1.13	−14	19.62 ± 0.58	20.64 ± 2.42	+31	+38
Acid soluble fraction (cpm/unit O.D. 260 nm)[b] $\times 10^{-3}$	12.64 ± 1.43	10.94 ± 0.69	−13	14.59 ± 1.26	14.22 ± 1.58	+33	+30
Alkaline hydrolyzate (cpm/mg DNA)[b] $\times 10^{-3}$	8.86 ± 0.44	8.17 ± 0.63	−8	13.76 ± 2.42	13.93 ± 3.74	+68	+70

Adrenalectomized rats were used 7–8 days after being operated upon. A single dose of aldosterone (5 μg/100 g body weight) was given intraperitoneally to adrenalectomized rats 1 hr or 2 hr prior to the isotope injection. All animals received an intraperitoneal injection of 6-[^{14}C]orotic acid (5 μCi/100 g body weight) 20 min before sacrifice.

[a] Number in parenthesis indicates the number of animals used.
[b] Mean ± standard error.

Table 2
Effect of adrenalectomy and aldosterone treatment on the incorporation of [^{3}H]cytidine into different fractions of rat kidneys.

Fraction	[a]Normal$_{(3)}$	[a]Adrenalectomized$_{(3)}$	Difference (%)	[a]Adrenalecomized plus aldosterone treated		Difference (%)	
				1 hr$_{(5)}$	2 hr$_{(4)}$	1 hr	2 hr
Total counts (cpm/mg DNA)[b] $\times 10^{-4}$	12.49 ± 1.32	9.36 ± 1.62	−25	14.05 ± 1.14	13.60 ± 0.67	+50	+45
Acid soluble fraction (cpm/unit O.D. 260 nm)[c] $\times 10^{-3}$	8.56 ± 0.98	8.12 ± 0.92	−4	10.94 ± 0.66	11.09 ± 0.48	+34	+35
Alkaline hydrolyzate (cpm/mg DNA)[b] $\times 10^{-3}$	9.76 ± 0.82	5.79 ± 0.42	−41	8.84 ± 0.95	9.01 ± 1.77	+53	+56

Adrenalecomized rats were used 7–8 days after being operated upon. A single dose of aldosterone (5 μg/100 g body weight) was given intraperitoneally to adrenalectomized rats 1 hr or 2 hr prior to the isotope injection. All animals received an intraperitoneal injection of 5-[^{3}H]cytidine (25 μCi/100 g body weight) 20 min before sacrifice.

[a] Number in parenthesis indicates the number of animals used.
[b] Mean ± standard error.

acid soluble pool:DNA ratio were 13.98 ± 0.33 for normal, 13.55 ± 0.34 for adrenalectomized and 14.61 ± 0.29 for adrenalectomized aldosterone treated animals (values are A_{260} units/mg DNA).

We examined the incorporation of three different radioactive precursors, [^{3}H]uridine, [^{3}H]cytidine and [^{14}C]orotic acid in the hope of finding a situation where there was a specific effect on incorporation into acid insoluble material. Of these precursors, [^{3}H]uridine is incorporated to an extremely limited extent (data not shown) and, as was shown above, the major effect for [^{3}H]cytidine and [^{14}C]orotic acid is on precursor uptake and hence on the specific activity of the soluble pool. This means that the previously observed effect [2, 4, 5] of aldosterone on precursor incorporation does not necessarily indicate a stimulation of RNA synthesis. However, this cannot be ruled out, particularly in the light of data showing a stimulation of RNA polymerase following hormone treatment [14, 15].

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